Annals of Phytomedicine 4(1): 61-67, 2015

Journal homepage: www.ukaazpublications.com

ANNALS OF PHYTOMEDICINE
An International Journal

ISSN : 2393-9885

Phytochemical investigations and characterization of antimicrobial activity of bioguided fractionated leaves of *Agave americana* L.

M.P. Babu, Raman Dang* and Kuntal Das*

Al-Ameen College of Pharmacy, Department of Pharmacognosy and Phytochemistry, Hosur Road, Bengaluru-560027, Karnataka, India
**Department of Pharmacognosy and Phytochemistry, Krupanidhi College of Pharmacy, #12/1, Chikka Bellandur, Carmelaram Post, Varthur Hobli, Bengaluru-560035, Karnataka, India*

Received March 10, 2015: Revised March 30, 2015: Accepted April 5, 2015: Published online June 30, 2015

Abstract

The aim of the present study is to evaluate the phytoconstituents details and the potentiality of antimicrobial effect of the leaves extracts of *Agave americana* L. (AA) plant that are successively extracted with various solvents, *viz*. petroleum ether, chloroform, methanol and water. Preliminary study revealed the presence of steroidal saponins along with protein, amino acids and other essential oils. The presence of antimicrobial activity against two gram positive (*Bacillus substilis* NCIM 2439, *Staphylococcus aureus* NCIM 2079) and two gram negative organisms (*Escherichia coli* NCIM 2065, *Pseudomonus aeruginosa* NCIM 2200) was carried out for all the extracts, using nutrient agar plate method and compared with standard chloramphenicol (100 mg/ml). Based on MBC value, the concentrations were selected for the study. Methanolic extract showed better activity at concentration ranges of 50 to 202 mg/ml, than other extracts but the activity was lesser than that of standard. Further, bioautography of fractionated compounds confirmed the same at Rf 0.42. Thereafter, methanolic extract was subjected to bioguided fractionation and column chromatography to isolate new compound from purified precipitation of n-butanol fraction that confirmed the antimicrobial activity, due to presence of saponin, *i.e*. (25R)-3 beta, 6 alpha-dihydroxy-5alpha-spirostan-12 one 3,6-di-O-beta-D-glucopyranoside which was supported by HPLC, NMR, IR MASS study.

Key words: Agave, bioguided fraction, antimicrobial study, MBC, isolation

1. Introduction

Plants based natural products have been used as curative agents from long days for variety of ailments in all over the world because herbal products play an important role in drug development in the pharmaceutical field. *Agave americana* L. (AA) is most commonly grown as an ornamental plant and cultivated worldwide, belongs to the family, Agavaceae. It is also known as American aloes or century plant (Anonymous, 2008). The plant is stout rhizomatous with aerial stem concealed by the leaf base. It has long, fleshy, rigid, hard, spinously toothed and lanceolate leaves, which grow directly out from the central stalk to form a dense rosette (Kirtikar and Basu, 1989; Rizwan *et al*., 2012). The plant is native of Mexico and well distributed throughout the world mainly in tropical countries like America, Kenya, Tanzania, Cuba, Africa and Asia (Lewin and Pearce, 1985). In India, the plants are grown near to railway line, road sides and river banks and as a hedge plant in dry land areas besides the plants are cultivated in South India, mainly in Andhra Pradesh, and also in Uttar Pradesh and Rajasthan (Singh *et al*., 2014). Literature survey revealed the presence of steroidal saponins, and other sapogenins in the leaves of Agave (Kintja *et al*., 1975). Further, Rastogi and Mehrohtra (1980) have reported the presence of ten steroidal saponins, *viz*. Agaveside A, B, C, C', D, E, F, G, H and I. There are different saponins that are isolated and identified from AA such as hecogenin, tigogenin, agavasaponin E and H (Yokosuka *et al*., 2000; Jin *et al*., 2003; Tewari *et al*., 2014). Data in the literature indicate that hecogenin and tiogenin isolated from AA, induce greater anti-inflammatory activity than the aqueous extract (Peana *et al*., 1997). Furthermore, Mana *et al*. (2010) found that the leaves have compounds with antitumor activity. AA extract orally administered to sheep, with different doses of saponins (120, 240 and 360 mg/kg) has antiprotozoal activity, as well as the capacity to lower the serum concentration of cholesterol and glucose (Nasri and Salem, 2012). The plant has antibacterial properties against *Staphylococcus* spp, *Pseudomonas aeruginosa* and *Escherichia coli* (Parmar *et al*., 1992; Khare, 2007; Menchaca *et al*., 2013; Kandhasamy and Vasudevan, 2015). Number of workers have explained about the extraction, isolation, purification and structural elucidation of the steroidal saponins from Agave (Singh and Perriera, 1963; Dixit *et al*., 1974; Mahoto *et al*., 1982), but extraction with bioguided fraction along with identification of compound in relation to microbioside activity was not revealed so far and further more in recent era, there is widespread interest in drugs, obtained from natural plant products for their potential antimicrobial activity due to less side effect and alternate source of antibiotics. Looking at that the present investigation has carried out with the aim of antimicrobial study of step wise fractionated extracts of AM leaves and confirmation of isolated compound responsible for the activity.

Author for correspondence: Professor Raman Dang
Principal, Krupanidhi College of Pharmacy, #12/1, Chikka Bellandur, Carmelaram Post, Varthur Hobli, Bengaluru-35, Karnataka, India
E-mail: dangraman2000@yahoo.co.in
Tel.: +91-9945846106, 8025527363

2. Materials and Methods

2.1 Collection and preparation of plant extracts

The leaves of AA were collected from Hosur, Karnataka and were authenticated by Dr. Yoga Narashima from RRI (Regional Research Institute, Bengaluru) and Dr. Gopinathan from Sami Labs, Bengaluru. The leaves samples were kept in herbarium in Al-Ameen College of Pharmacy, Bengaluru (Herbarium No: AACP/BABU/AGAVE-207/2002) for future reference. The leaf samples are dried at 70^0C in an oven and powdered by mechanical means and stored in closed container for further studies.

2.8 kg of oven dried powdered material was successively refluxed with pet ether followed by chloroform, methanol and water for 12 h. Each time before extraction, with the next solvent, the powdered material was air dried and further extracted. Finally the marc obtained was macerated with water for 12 h to get aqueous extract. All the extracts were concentrated with rotary flash evaporator at 45^0C and weighed. The yield of the extracts were calculated and tabulated. Resulting extracts were kept in small glass bottles with proper labeled and stored at refrigeration condition at 4^0C for further investigation.

2.2 Phytochemical analysis

The extracts were subjected to phytochemical analysis to detect the presence of biomolecules namely; alkaloids, glycosides, carbohydrates, phenols, tannins, saponins, fixed oils, fats and amino acids, using the standard qualitative procedures as described by Trease and Evans (1989).

2.3 Test microorganisms

Test bacteria, two gram positive (*Bacillus substilis* NCIM 2439, *Staphylococcus aureus* NCIM 2079) and two gram negative organisms (*Escherichia coli* NCIM 2065, *Pseudomonus aeruginosa* NCIM 2200) were procured from National Centre Institute of Microbiology, Mysore, Karnataka State. The bacteria were rejuvenated on nutrient agar medium at 37°C for 24 h and stocked at 4°C on nutrient agar slant. Subculture was prepared from the stock before the bioassay.

Determination of antibacterial activity by agar well diffusion method, 20 ml molten nutrient agar media were mixed with 1 ml of (10^6cfu/ml) each test bacteria and poured in sterile petri dishes separately. After complete solidification, 9 mm diameter wells were made using sterile cork borer and filled with 200 µl of different concentrations of petroleum ether, chloroform, methanol and aqueous extracts of agave leaves extracts. 100 mg of streptomycin was used as standard. Then the plates were incubated at 37^0 C for 24 h and antibacterial activity was determined by measuring the diameter of clear zone around the well.

2.4 Determination of Minimum Bactericidal Concentration (MBC)

Equal volume of the various concentration of each extract and Mueller Hinton broth was mixed in microtubes to make up 0.5 ml of solution. 0.5 ml of McFarland standard of the organism suspension was added to each tube (Bonjar, 2004). The tubes were incubated aerobically at 37°C for 24 h. Two control tubes were maintained for each test batch. These include tube-containing extract without inoculums and the tube containing the growth medium and inoculum. The MBC was determined by subculturing the test dilution on Mueller Hinton agar and incubated for 24 h. The highest dilution that yielded no single bacterial colony was taken as the MBC (Akinyemi *et al*., 2005).

Direct bioautography was used for the screening of antibacterial compounds and the Rf values for all the extracts were determined. The agar overlay method (immersion bioautography) was used for detection of antimicrobial compounds. TLC plate (Merck Silica Gel 60 F254) was loaded with 10 µl of solvent extracts in duplicate. The solvent system used was Butanol: acetic acid: water (4: 1: 5). The chromatogram was kept for evaporation of the solvent. Developed chromatogram was placed on sterile Muller Hinton agar plate (10 ml) for detection of antibacterial activity by spraying anisaldehyde sulphuric acid reagent. After agar got solidified, the petri plates were kept at 4°C for diffusion for 3 h. Plates were then incubated at room temperature for 24 h for bacterial culture (Singh and Dwivedi, 1991; Ncube *et al*., 2008).

2.5 Bioguided fractionation of the extract

Depends on the significant activity, methanol leave extract was selected for bioguided fraction extraction. Methanol extract of the leaves was preliminary treated with acetone and gave two parts, *i.e*. acetone soluble part and acetone insoluble part. Acetone insoluble part (*i.e*, extract in methanol) was further extracted with successive solvents, *viz*. n-hexane, n-butanol and aqueous. Thereafter depends on higher antimicrobial activity, n-butanol part was selected and further washed with acetone solution to remove the impurities. Acetone insoluble part was subjected to column chromatography using silica gel 60/120 and neutral alumina as adsorbent and various solvents based on polarity. Ultimately isolated the compound which was crystallized using methanol to get creamy powder. Below the schematic diagram revealed the detail study of bioguided fractionated extraction.

2.6 Characterization of the isolated compound

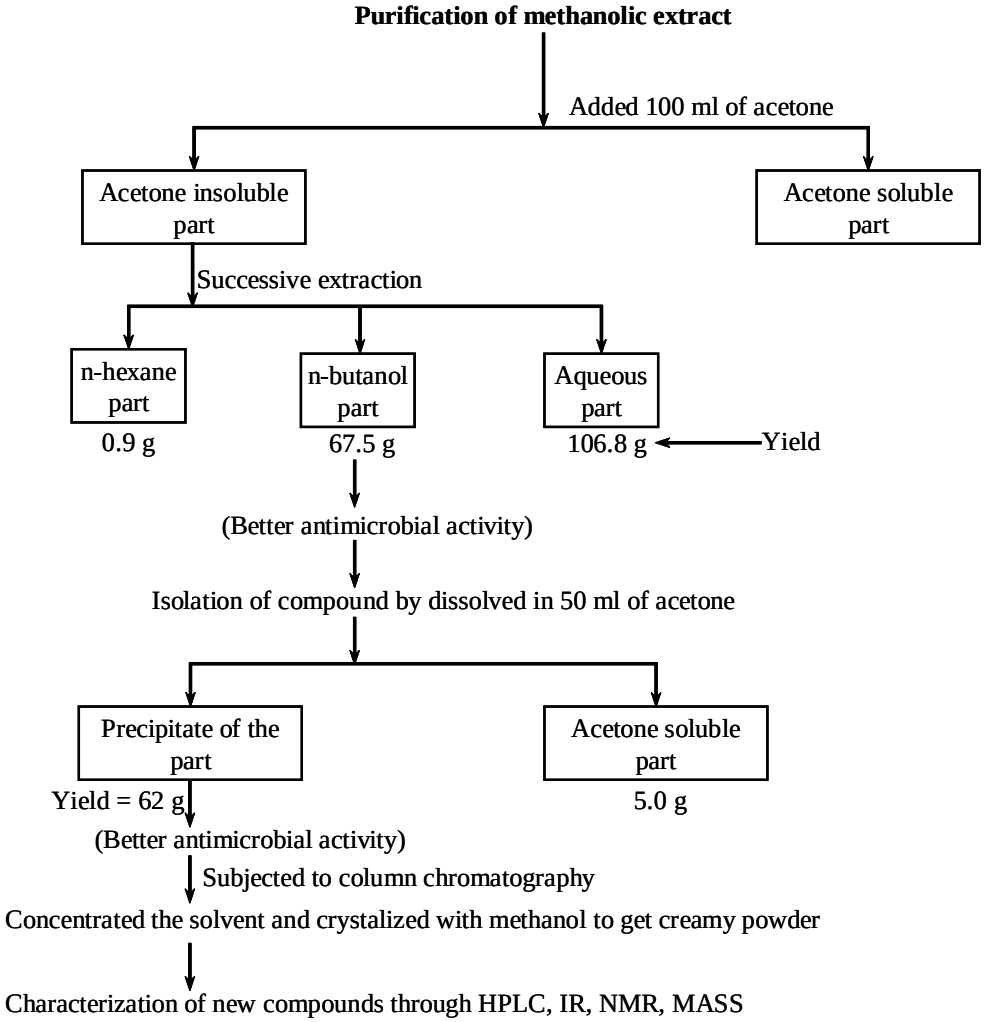
Isolated compound was quantified and identified by using HPLC, NMR, IR and MASS spectroscopy and the data were analyzed to identify the proposed structure of the isolated compound that was responsible for antimicrobial activity.

HPLC: Isolated compound was analyzed by using HPLC as per the method developed by Sami Labs, Bangalore. C_{18} column was used as stationary phase and Methanol: Water (80: 20) was used as mobile phase and detected at 210 nm with the flow rate of 1ml/min. 1 mg/ml concentration was prepared for both sample and standard by dissolve both of them separately in methanol.

IR spectroscopy: An IR spectrum of isolated compound was recorded by KBr pellets in range of 4000 - 500 cm^{-1} on a FTIR (Shimadzu 8700) at Al-Ameen College of Pharmacy, Bangalore. The frequency was reported in wave numbers (cm^{-1}).

NMR: There are two different NMRs are recorded namely; ^{13}C-NMR and ^{1}H- NMR. Isolated compound in pyridine was recorded in NMR, using AMX 400 spectrophotometer at Astra-Zeneca, Bangalore. Chemical shifts (d) are reported in parts per million (ppm) downfield from internal reference Tetramethylsilane (TMS).

MASS spectroscopy: MASS spectroscopy of isolated compound in methanol was recorded in LC-MSD-Trap 00148, spectrophotometer at Biocon India Ltd, Bangalore with their principle molecular fragments.

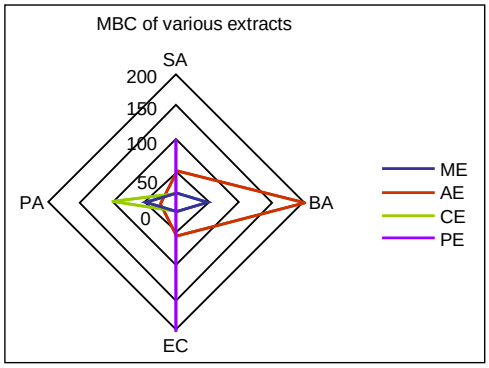


3. Results and Discussion

Successive solvent extraction of the leaves of AA resulted an aqueous extract was crystalline in nature whereas methanolic extract was hygroscopic and remaining two extracts were sticky in nature. The qualitative test for the presence of phytochemicals revealed that the methanol and aqueous extracts of agave possess maximum phytochemicals whereas pet ether and chloroform extract showed presence of very few constituents like phytosterol, fats and oil, were present in pet ether extract whereas small quantities of saponin and fats and oils were presence in chloroform extract. Saponins, carbohydrates, glycosides, phytosterol and amino acids were present in methanol and aqueous extracts. Unfortunately, phenols and tannins were not present in any of the tested extracts. The presence of saponins and sterols in AA was reported in literature and they are the active antimicrobial constituents among the constituents (Krithikar and Basu, 1989; Garcia *et al*., 1998; Panghal *et al*., 2011).

High percentage of yield was obtained from aqueous extract (20.2%), followed by methanol (15.5%), petroleum ether (3.5%) and chloroform extract (1.4%). But methanol extracts revealed higher inhibitory effects on tested organisms compared to other extracts. It indicates that the inhibitory effect does not depend on the yield percentage. Thereafter the MBC was recorded for all the extracts and revealed methanolic extract showed maximum activities against all the organisms with a concentration ranges of 12.5-50 mg/ml whereas aqueous extract showed activity at 25-200 mg/ml for all but chloroform and pet ether extract did not show activity against all but all the extracts were significantly active against *E. coli* (Figure 1) and, hence, the methanol and aqueous extracts were able to inhibit the growth of both microbes while the petroleum ether and chloroform extract failed to inhibit or very less growth of microbes. Out of four extracts, methanolic extract showed better inhibitory effect on *E. coli* (28 ±0.012, at 202 mg/ml conc.), followed by *S. aureus* (20 ±0.020, at 100 mg/ml conc.), but the effects were concentration dependent (Das *et al*., 2009; Sammaiah *et al*., 2006) and the values were lesser than the standard chloramphenicol (33 ±0.011, at 100 mg/ml conc.). These results further substantiate the fact that saponins present as an active phytoconstituent in methanol extract (Jinukuti and Giri, 2013). Further the activity was confirmed with the bioautography at calculated Rf value of 0.42. (Figure 2). Further screening of comparative antimicrobial activity of purified fractionated methanolic extract was carried out and revealed precipitated n- butanol fraction showed higher antimicrobial effect in *E coli* (32.7 ± 0.221), followed by *P. aeruginosa* (30.2 ± 0.121) at concentration of 201.2 mg/ml. (Table 1).

Figure 1: Minimum bacterial concentration of extracts



SA = *Staphylococcus aureus*; **BA** = *Bacillus substilis*;
EC = *Escherichia coli*; **PA** = *Pseudomonus aeruginosa*
ME = Methanolic extract; **AE** = Aqueous extract;
CE = Chloroform extract; **PE** = Pet ether extract

Figure 2: Bioautography of methanolic extract

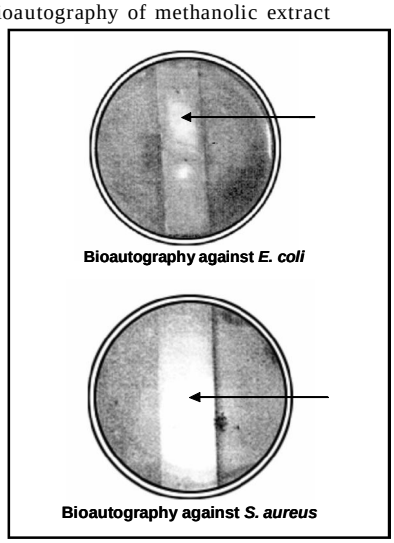


Table 1: Comparative antimicrobial screening of isolated selected fractions

Extracts	Conc taken mg/ml	Conc per bore mg/ml	Zone of inhibition (mm ±SEM)			
			SA	PA	EC	BS
n-butanol fraction (405.4 mg + 2 ml sterile water)	203.2	40.4	12 ±0.010	13 ± 0.110	14 ± 0.021	13 ± 0.001
	102.1	20.2	10 ± 0.121	10 ± 0.130	12 ± 0.120	10 ± 0.020
	50.5	10.1	Nil	Nil	Nil	Nil
Purified ppt of n-butanol fraction 403.4 mg + 2 ml of sterilized water	201.2	40.4	23 ± 0.211	30.2 ± 0.121	32.7 ± 0.221	20 ± 0.211
	101.7	20.2	20 ± 0.210	25 ± 0.111	24 ± 0.030	15 ± 0.010
	50.04	10.1	16 ± 0.121	20 ± 0.100	20 ± 0.021	10 ± 0.130
ppt of n-butanol fraction 401.4 mg + 2 ml of sterilized water	201.5	40.4	20 ± 0.200	18 ± 0.110	15 ± 0.201	16 ± 0.033
	100.1	20.2	14 ± 0.100	12 ± 0.111	14 ± 0.020	10 ± 0.020
	50.5	10.1	10 ± 0.121	Nil	10 ± 0.001	Nil
Chloramphenicol	100	20	33 ± 0.001	Nil	33 ± 0.001	38 ± 0.011

Plates were triplicated; **SA** = *Staphylococcus aureus*; **BA** = *Bacillus substilis*; **EC**= *Escherichia coli*;
PA= *Pseudomonus aeruginosa;* **Nil** = No inhibition

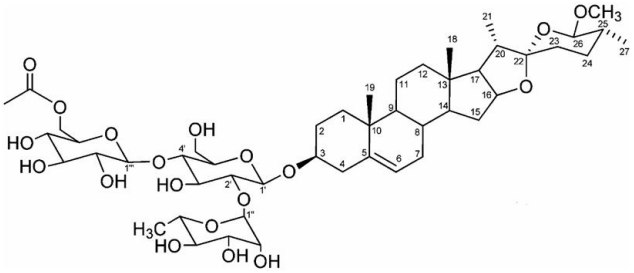
Isolated compound was subjected to HPLC analysis and the chromatogram revealed a distinct peak at Rt of 6.85 minute along with two other peaks showed in Graph 1. Further, UV data, characteristic IR andNMR (^{1}H and ^{13}C) spectral peaks of compound was found to be super imposable with the reported compound and spectra are depicted in Table 2.

Table 2: Spectral studies of isolated compound

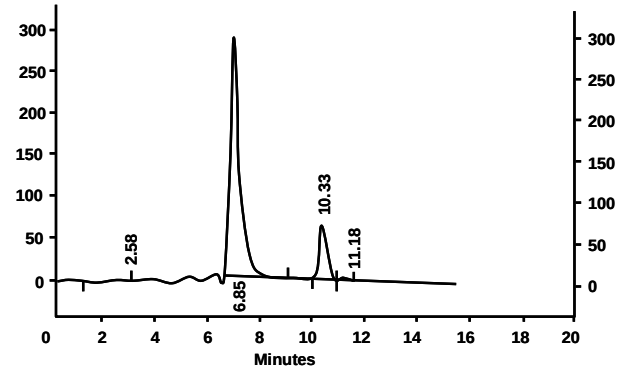
Name of compound	UV light absorption band	IR KBr: vcm^{-1}/ max	^{1}H NMR (500 MHz, CD_3 OD, δ ppm)	^{13}C NMR (125 MHz, CD_3 OD, δ ppm)
Bisdesmosidicspirostanol saponins	207 nm	OH, 3419 cm^{-1}; CH, 2929 cm^{-1}; C=C,1643 cm^{-1}; C=O, 1737 cm^{-1}	1.30 (H_1eq), 0.70 (H_1 ax); 2.00 (H_2eq), 1.42 (H_2 ax); 3.92 (H_3); 3.42 (H_4eq), 1.41 (H_4 ax); 1.20 (H_5); 3.65 (H_6); 2.60 (H_7eq), 1.10 (H_7 ax); 1.83 (H_8); 0.78 (H_9); 2.20 dd (H_{11}eq), 2.31 (H_{11} ax); 1.28 (H_{14}); 2.00 (H_{15}); 2.60 dd (H_{17}); 1.04 ss (H_{18}); 0.92s (H_{19}); 1.83 (H_{20}); 1.38 (H_{21}); 1.64 (H_{23}eq), 1.60 (H_{23} ax); 1.53 (H_{24} 2H); 0.72 d, 5.12 d, 4.04 d, 4.23 d ($H_{27,}$ 1', 2', 3')	36.6 (C_1eq); 28.5 (C_2eq); 75.6 (C_3); 28.5 (C_4); 50.8 (C_5); 79.9 (C_6); 41.3 (C_7); 33.9 (C_8); 53.8 (C_9); 36.6 (C_{10}); 37.5 (C_{11}); 212.5 (C_{12}); 56.3 (C_{13}); 56.2 (C_{14}); 31.7 (C_{15}); 79.9 (C_{16}); 53.8 (C_{17}); 16.9 (C_{18}); 13.3 (C_{19}); 41.5 (C_{20}); 13.3 (C_{21}); 106.3 (C_{22}); 31.7 (C_{23}); 28.5 (C_{24}); 31.7 (C_{25}); 67.4 (C_{26}); 16.9, 101.6, 75.6, 78.5, 71.7 ($C_{27,}$ 1', 2', 3', 4')

FT-IR spectroscopy data interpretation indicated the presence of major functional groups at peaks of various positions (Graph 2) and predicted as the isolated compound may be beta-D-glycopyranosyl residues in steroidal di-glucosides. The NMR study further revealed the isolated compound is a steroid di-glycoside.

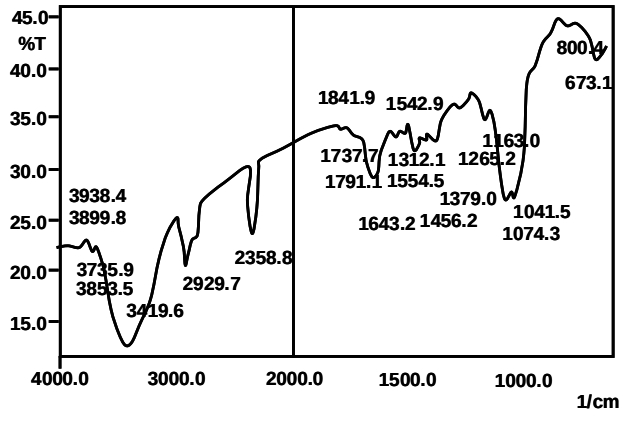
^{1}H-NMR data predicted that isolated compound in pyridine contains 2,3 proton singlets at δ = 1.05 and 0.76; 2, 3 proton doublets at δ = 1.34 and 0.72 and two anomeric proton singlets at δ = 5.10 and 4.90. Further ^{13}C-NMR data revealed the same with respect to absorbing anomeric proton singlets at δ = 5.10 and 4.90 to the C-3 and C-6 resonances. Finally MASS analysis was carried out using LC-MSD-Trap 00148 to ascertain the molecular weight and was found to be 795.1 (Graph 3). The overall repots summarized the isolated compound to be Bisdesmosidicspirostanol saponin, *i.e.* (25R)-3 beta, 6 alpha- dihydroxy- 5 alpha-spirostan-12 one 3,6-di-O-beta-D-glucopyranoside and the data are consistent with the structure reported by the Mimaki *et al.* (2000) and the structure is as follows:



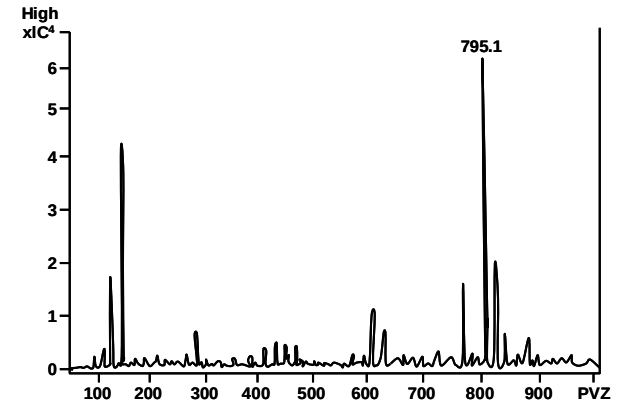
Graph 1: HPLC of isolated compound by PDA method



Graph 2: FT-IR spectrum of isolated compound



Graph 3: MASS spectroscopy of isolated compound



4. Conclusion

This study has proved that leaf extracts of agave show antibacterial activity and the presence of various phytochemical constituents mainly saponins. The bioautography is a preferred tool in detecting the presence of antimicrobial compounds in extracts at the earliest stages of down streaming and screening of antimicrobial compounds to the findings that the isolate produces antimicrobial compound, *i.e.* (25R)-3 beta, 6 alpha- dihydroxy- 5 alpha-spirostan-12 one 3,6-di-O-beta-D-glucopyranoside which was established by HPLC, IR, NMR, and MASS spectroscopy data. Further confirmation needs to done by ORD and X-ray crystallography and to formulate as various topical antimicrobial products.

Acknowledgements

The authors are thankful to the Sami Labs, Bangalore for method development of HPLC study, Astra-Zeneca, Bangalore for providing NMR study, Biocon India Ltd., Bangalore for MASS study and finally to our Principal, Al-Ameen College of Pharmacy for providing IR study and other facilities to fulfill the project.

Conflict of interest

We declare that we have no conflict of interest.

References

Akinyemi, K.O.; Oladapo, O.; Okwara, C.E.; Ibe, C.C. and Fasure, K.A. (2005). Screening of crude extracts of six medicinal plants used in southwest Nigerian unorthodox medicine for antimethicillin resistant *Staphylococcus aureus* activity. BMC Complementary and Alternative Medicine., **5**:6.

Anonymous (2008) Agaves; Retrieved March 15, 2015 from http:// .www en.wikipedia.org/wiki/Agave_americana.

Bonjar Shahidi, G.H. (2004). Evaluation of antibacterial properties of Iranian medicinal plants against *Micrococcus aureus, Serratia marcescens, Klebsiella pneunomiae* and *Bordella bronchoseptica*. Asian Journal of Sciences, **3**(1):82-86.

Das, K.; Dang, R. and Gupta N. (2009). Comparative antimicrobial potential of different extracts of leaves of *Stevia rebaudiana* Bert. International Journal of Natural and Engineering Sciences., **3**(1):65-68.

Dixit, B.S.; Khanna, R.K.; Srivatsava, S.N. and Kapoor, C.D. (1974). Isolation of hecogenin from some agave species. Ind. J. Pharm., **36**:117-120.

Garcia, M.D.; Farnandez, M.A.; Saenz, M.T. and Quilez, A. (1998). Antibacterial activity of *Agave intermixta* and *Cissussicyoides*. Fitoterpia, **70**:71-73.

Jin, J.M.; Liu, X.K. and Yang, C.R. (2003). Three new hecogenin glycosides from fermented leaves of *Agave americana*. J. Asian Nat. Prod. Res., **5**:95-103.

Jinukuti, M.G. and Giri, A. (2013). Antimicrobial activity of phytopharmaceuticals for prevention and cure of diseases. Ann. Phytomed., **2**(2):28-46.

Kandhasamy, R. and Vasudevan, K. (2015). Development of *Agave americana* antibacterial fabric for healthcare applications. International Journal of Innovative Research in Science, Engineering and Technology., **4**(3):848-851.

Khare, C.P. (2007). Indian Medicinal Plants. An Illustrated Dictionary, Springer Science and Business Media, pp:710.

Kintja, P.K.; Bobejko, W.A.; Krochmaljuk, W.W. and Tschirva, W.J. (1975). Uber die saponineindenblattern von *Agave americana* Linn. Pharmazie, **31**:396-397.

Kirtikar, K.R. and Basu, B.D. (1989). Indian medicinal plants, MIS periodical experts, New Delhi, pp:2466.

Lewin, M. and Pearce, E.M. (1985). Handbook of Fiber Sciences and Technology IV, Fiber Chemistry, Marcel Dekker, NY.

Mahato, S.B., Ganguly, A.N. and Sahu, N.P. (1982). Steroidal saponins. Phytochemistry, **21**:959-978.

Mana, S.; Gaoa, W.; Zhang, Y.; Huangc, L. and Liud, C. (2010). Chemical study and medical application of saponins as anticancer agents. Fitoterapia, **81**:703-714.

Menchaca, M.C.V.; Morales, C.R.; Star, J.V.; Cárdenas, A.O.; Morales, M.E.R.; Gonzalez, M.A.N. and Gallardo, L.B.S. (2013). Antimicrobial activity of five plants from Northern Mexico on medically important bacteria. African Journal of Microbiology Research, **7**(43):5011-5017.

Mimaki, Y.; Yokosuka, S. and Sashida, Y. (2000). A new steroidal saponin from the leaves of *Agave americana*. Planta Med., **66**:393.

Nasri, S. and Salem, B. (2012). Effect of oral administration of *Agave americana* or *Quillaja saponaria* extracts on digestion and growth of barberine female lamb. Livest. Sci., **147**:59-65.

Ncube, N.S.; Afolayan, A.J. and Okoh, A.I. (2008). Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. African Journal of Biotechnology, **7**(12):1797.

Panghal, M.; Kaushal, V. and Yadav, J.P. (2011). *In vitro* antimicrobial activity of ten medicinal plants against clinical isolates of oral cancer cases. Annals of Clinical Microbiology and Antimicrobials, 10:21.

Parmar, V. S.; Jha, H. N.; Gupta, A. K. and Prasad, A. K. (1992). Agamanona, a flavanona from *Agave Americana*. Phytochemistry, **31**(7):2567-2568.

Peana, A.; Morettin, M.;Manconi, V.;Desole, G. and Pippia, P. (1997). Anti-inflammatory activity of aqueous extracts and steroidal sapogenins of *Agave americana*. Planta Med., **63**:199-202.

Rastogi, P.R. and Mehrotra, B.N. (1980). Compendium of Indian medicinal plants. Publication and Information Directorate, New Delhi. pp:22-25.

Rizwan, K.; Zubair, M.; Rasool, N.; Riaz, M.; Zia-UlHaq M. and de Feo, V. (2012). Phytochemical and biological studies of *Agave attenuata*, International Journal of Molecular Sciences, **13**(5): 6440-6451.

Singh, H.K. and Pereira, W. (1963). Chemical examination of the leaves of *Agave americana* Linn. Ind. J. Chem., **2**:297-298.

Singh, R.K. and Dwivedi, R.S. (1991). Ecology and biology of *Sclerotium rolfsii* Sacc. International Journal of Tropical Plant Diseases, **9**:161-171.

Sammaiah, G.; Srivastava, R.S. and Ascervadam, T. (2006). Antmicrobial activity of whole plant extract of *Polygala erioptera*. Indian J. Nat. Prod., **22**(2):31-33.

Singh, R.K.; Mittal, P.K.; Kumar, G. and Dhiman, R.C. (2014). Evaluation of mosquito larvicidal efficacy of leaf extract of a cactus plant, *Agave sisalana*. Journal of Entomology and Zoology Studies, **2**(1):83-86.

Tewari, D.; Tripathi, Y.C. and Anjum, N. (2014). *Agave sislana*: a plant with high chemical diversity and medicinal importance. World Journal of Pharmaceutical Research., **3**(8):238-249.

Trease, G.E. and Evans, W.C.(1989). Phytochemical screening. *In*: Textbook of Pharmacognosy, Trease, G.E. and W.C Evans (Eds.). 10th Edn.,bailiere Tindal Limited, London, pp:541.

Yokosuka, A.; Mimaki, Y.; Kuroda, M. and Sashida, Y. (2000). A new steroidal saponin from the leaves of *Agave americana*. Planta Med., **66**:393-396.